A Comprehensive Metagenomic Analysis of Bacterial and Fungal Microbiome Responses to Leaf-Based Compost Amendment in Soil, Unveiling the Bio-Fertilizing Potential

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Abstract

A comprehensive understanding of soil microbiome dynamics is imperative for bolstering sustainable agricultural productivity and devising effective soil management strategies. This study investigates the impact of leaf and other compost amendments on soil microbial richness and diversity. Metagenomic profiling techniques targeting 16S rRNA genes and Internal Transcribed Spacer (ITS) region were employed to examine the bacterial and fungal microbiome structure in both pre-plantation and post-harvest soils. The findings reveal a notable increase in beneficial bacterial and fungal genera in the soil amended with compost, including *Bacillus*, *Nitrospira*, *Planctomyces*, *Myxococcus*, *Agromyces*, *Wallemia*, *Pichia*, and *Microascus*. Conversely, pathogenic genera such as *Corynebacterium*, *Burkholderia*, *Nocardia*, *Olpidium*, *Penicillium*, *Acremonium*, and *Alternaria* exhibited higher abundance in soil amended with chemical fertilizers, highlighting the potential of bio-compost amendments in bioremediation and pathogen control. The post-harvest soil samples amended with leaf-based compost showed an increase of 116% in beneficial bacterial genera and a 21% increase in beneficial fungal genera, accompanied by a 59% and 60% decrease in pathogenic bacterial and fungal genera, respectively.

In contrast, the chemical fertilizer amendment reduced beneficial bacterial and fungal genera by approximately 49% and 2%, respectively, while increasing pathogenic bacterial genera by about 132% in the post-harvest soil. The study underscores the significant impact of leaf-based bio-compost amendments on soil microbial richness, diversity, and overall soil health. Leaf-based bio-compost enhanced microbial diversity and functionality, fostering beneficial microorganisms that play pivotal roles in nutrient cycling, plant growth promotion, and strengthening soil ecosystem resilience.

Introduction

Investigating soil microbiomes and their dynamic interactions has gained considerable interest, primarily because they are crucial in maintaining soil health, facilitating nutrient cycling, and influencing plant productivity. The structure, composition, and diversity of microbial communities in soil are crucial for maintaining ecosystem functions and influencing plant growth [1–3]. One strategy to enhance soil microbiome activity and function involves the application of organic composts derived from various sources such as leaf litter, animal manures, and food waste [4]. Organic compost, a vital soil amendment, enriches the soil with essential nutrients, improves soil structure and water retention, fosters beneficial microbial activity, and aids in pH balancing. It also mitigates soil erosion, reduces compaction, and promotes moisture retention, reducing the need for synthetic fertilizers [5, 6].

Furthermore, organic composts can alter the structure and composition of soil microbial communities, leading to changes in microbial diversity, functions, and activity [7, 8]. Using organic waste to produce compost, we nourish plants, reduce landfill waste, and support a sustainable environment. It is a natural, eco-friendly way to enhance soil health, encourage plant growth, and contribute to a more sustainable approach to agricultural farming. Understanding the impact of bio-composts on the soil microbiome
dynamics is essential for optimizing their application in agriculture and sustainable land management practices.

Several studies have reported the benefits of biocomposts to soil health and plant growth, such as increased nutrient solubilization, plant growth promotion, and plant pathogen suppression [9, 10]. These reports also highlighted the potential of bio-compost formulations in shaping soil microbiomes for agricultural systems. Compost could alter the structure of the microbial community and introduce new microorganisms into the soil system. In addition to the studies above, other investigations have contributed to our understanding of the microbiome dynamics in soils amended with bio-composts. Bio-compost application could induce significant changes in microbial community structure and enzymatic activities in agricultural soils [11, 12].

Furthermore, a study by Samaddar [13] examined the impact of bio-compost amendments derived from different animal manures on soil microbial community composition and found distinct responses in microbial diversity and functional profiles. The interconnection between soil microbiomes, cultivars, food safety, and human health underscores the potential of beneficial microorganisms in the soil to improve environmental sustainability, food quality, and human health [14]. Soil health enhancement has become paramount for sustainable productivity, with improved food quality that can promote human health.

While previous research has explored the effects of bio-compost amendments on soil microbiomes, there is a need for further investigation into the specific dynamics and mechanisms involved. This research employs 16S rRNA and ITS metagenomic profiling techniques to examine the dynamics of microbiomes in soils before planting and after harvest, following the application of various bio-composts. The Internal Transcribed Spacer (ITS) region and 16S rRNA gene serve as molecular markers to identify and characterize bacterial and fungal communities, utilizing phylogenetic relationships as a basis. [15]. The 16S and ITS metagenomics offer distinct advantages over ordinary high-throughput sequencing (HTS) in microbial community analysis. Targeting the 16S ribosomal RNA (rRNA) gene and ITS region, the 16S and ITS metagenomics allows for a focused examination of bacterial and fungal communities and diversity within a sample [16]. This targeted approach simplifies taxonomic classification and phylogenetic analysis and facilitates comparative studies across different samples, making it cost-effective compared to whole-genome sequencing. The amplification and sequencing of conserved regions in the 16S rRNA gene and ITS region ensure broad coverage and aid in biomarker discovery associated with specific microbial communities. The reliance on extensive 16S rRNA gene and ITS databases enables accurate taxonomic assignment and annotation of sequences.

Additionally, 16S and ITS metagenomics offer a faster data analysis turnaround time, making them powerful tools for researchers investigating bacterial and fungal diversity, especially in microbial ecosystems [17]. The metagenomic profiling conducted in this study allows for a comprehensive analysis of microbial composition and diversity in the soil samples, helping identify beneficial and pathogenic microorganisms. By comparing pre-plantation soils amended with different composts to post-harvest soils, the study assesses the temporal impact of bio-compost amendments on soil microbiome structure.
This analysis provides insights into changes in microbial community structure, diversity, and potential shifts in functional profiles. Moreover, it helps identify specific microbial taxa influenced by bio-compost applications, potentially associated with improved soil fertility and plant productivity.

The novelty of this study lies in focusing on specific types of bio-composts derived from novel sources, such as leaf litter waste, and comparing their impact with various other organic waste composts, including cow dung manure, kitchen waste compost, municipal organic waste compost, and vermicompost. The study investigates the effects of these bio-composts on soil microbiome richness and diversity. Additionally, we analyze the temporal dynamics of the microbiome from pre-plantation to post-harvest stages, providing an understanding of the long-term effects of bio-compost amendments on soil microbial communities. This knowledge contributes to developing sustainable agricultural practices to improve soil health, nutrient recycling, and overall agricultural productivity.

Materials and methods

Experimental setup, sample collection and preparation

Two soil types, floodplain soil from the Yamuna riverbank near the Usmanpur area (28°41'48"N 77°12'38"E) and residential soil from the University of Delhi's North Campus (28°41'16"N 77°12'19"E), were collected (Fig. 1). Floodplain soil is of alluvial type, while residential soil is sandy. These soils were mixed with leaf-based compost and various other composts (cow dung manure, kitchen waste compost, municipal organic waste compost, and vermicompost) in a 5:1 ratio (w/w) before potting. We also set up the control pots with soil and chemical fertilizers without composts. The physicochemical qualities of composts were reported in a prior study [18]. The diammonium phosphate (DAP), calcium, and phosphate chemical fertilizers were mixed with soil at 4:1:1 in grams per kilogram of soil. We collected about 500 g of each sample of the pre-plantation soils in a sterilized zip-lock polybag. The plantation was done by potting red amaranth (Amaranthus cruentus) using these soils in different pots. Five kilograms (kg) of soil were taken in each pot for seedling and potting. The seedling and potting were done in April in an ambient environment and harvested in the first week of July. The average temperature during the growing period was 29ºC to 33ºC, and the humidity was 29–46%. We collected 500g of soil samples from each pot after harvest soil in a sterilized zip-lock polybag using a spatula from about 8 to 12 cm depth. The same compost amendment soil samples were mixed and homogenized adequately for further analysis (Table 1).

The physicochemical parameters of soils

The soil sample's electrical conductivity (EC) and Soil pH were analyzed using a pH meter and an EC meter. We analyse the samples by dissolving the soil in distilled water at a ratio of 1:2 (soil weight to distilled water volume). Then, we measure the pH and EC using the pH and EC meter. The soils' total organic carbon, nitrogen and sulphur content was analyzed using a CHNS analyzer (varioEL cube, Ser.no: 19171021). We analysed all the samples in triplicates and reported the mean value [19].
DNA Extraction and PCR Amplification of V3-V4 Region of 16s Gene:

For the next-generation sequencing, DNA extraction was done using the suitable method for the sample type from commercially available kits such as QIAGEN (Qiagen India Pvt Ltd, Delhi, India), ZYMO RESEARCH (California, USA), and Thermo-Fisher (Massachusetts, USA). DNA extraction was done as per the manufacturer's recommendation. To check the quality of the DNA before the PCR amplification, extracted DNA from the samples was subjected to NanoDrop and GEL Check before being taken for PCR amplification, with 2% agarose gels using a ladder of 1000 base pairs. The NanoDrop readings of 260/280 at 1.8 to 2 were used to determine the DNA's quality [20, 21].

For conducting the metagenomic analysis, the isolated DNA underwent amplification and sequencing to obtain the DNA sequences unique to both the V3-V4 region of the bacterial 16S rRNA gene and the Internal Transcribed Spacer Regions 1 to 4 (ITS 1–4) of the fungal genome [22, 23]. The amplification process employed a PCR mix comprising High-Fidelity DNA Polymerase, 0.5mM dNTPs, 3.2mM MgCl₂, and PCR Enzyme Buffer. For the 16S metagenomic analysis, the utilized primers were V34F: − 5'AGAGTTTGATGMTGGCTCAG3' and V34F: − 5'TTACCGCGGCMGCSGGCAC3'. Meanwhile, the primers for the ITS metagenomic analysis were ITS: − 5'TTGGTCATTTAGAGGAAGTAA3' and ITS2: − 5'GCTGCGTTCTTCATCGATGC3' [24]. The polymerase chain reaction (PCR) amplification conditions included 40ng of extracted DNA and 10 pM of each primer. The first step of denaturation was established at 95°C. Subsequently, 25 cycles were executed under the subsequent conditions: denaturation at 95°C for 15 seconds., annealing at 60°C for 15 seconds., elongation at 72°C for 2 minutes, and a concluding extension at 72°C for 10 minutes, succeeded by a maintenance period at 4°C. The resulting 16S and ITS PCR products underwent purification. They were subjected to gel electrophoresis using a 2% agarose gel and a 1000 base pair (bp) ladder, followed by a Nanodrop quality control assessment.
Table 1
Soil sample code along with its descriptions and the International Nucleotide Sequence Database Collaboration (INSDC) accession number of the 16S and ITS metagenomics

<table>
<thead>
<tr>
<th>Samples Code</th>
<th>Sample Group</th>
<th>Soil Type</th>
<th>Amendment</th>
<th>The International Nucleotide Sequence Database Collaboration (INSDC) accession number</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>16S ITS</td>
</tr>
<tr>
<td>YC</td>
<td>1</td>
<td>Pre-plantation floodplain soil</td>
<td>Cow dung manure</td>
<td>ERS15529941 ERS16534118</td>
</tr>
<tr>
<td>YD</td>
<td>1</td>
<td>Pre-plantation floodplain soil</td>
<td>Leaf waste compost</td>
<td>ERS15529942 ERS16534121</td>
</tr>
<tr>
<td>YK</td>
<td>1</td>
<td>Pre-plantation floodplain soil</td>
<td>Kitchen waste compost</td>
<td>ERS15529947 ERS16534536</td>
</tr>
<tr>
<td>YM</td>
<td>1</td>
<td>Pre-plantation floodplain soil</td>
<td>Municipal organic waste compost</td>
<td>ERS15530011 ERS16534120</td>
</tr>
<tr>
<td>YV</td>
<td>1</td>
<td>Pre-plantation floodplain soil</td>
<td>Vermicompost</td>
<td>ERS15530204 ERS16534119</td>
</tr>
<tr>
<td>YF</td>
<td>1</td>
<td>Pre-plantation floodplain soil</td>
<td>Chemical fertilizer</td>
<td>ERS15532765 ERS16534539</td>
</tr>
<tr>
<td>YCRAH</td>
<td>4</td>
<td>Post-harvest floodplain soil</td>
<td>Cow dung manure</td>
<td>ERS15532766 ERS16534535</td>
</tr>
<tr>
<td>YDRAH</td>
<td>4</td>
<td>Post-harvest floodplain soil</td>
<td>Leaf waste compost</td>
<td>ERS15532767 ERS16534540</td>
</tr>
<tr>
<td>YKRAH</td>
<td>4</td>
<td>Post-harvest floodplain soil</td>
<td>Kitchen waste compost</td>
<td>ERS15532768 ERS16534699</td>
</tr>
<tr>
<td>YMRAH</td>
<td>4</td>
<td>Post-harvest floodplain soil</td>
<td>Municipal organic waste compost</td>
<td>ERS15532770 ERS16539415</td>
</tr>
<tr>
<td>YVRAH</td>
<td>4</td>
<td>Post-harvest floodplain soil</td>
<td>Vermicompost</td>
<td>ERS15532769 ERS16539420</td>
</tr>
</tbody>
</table>
### Overview of sequencing and bioinformatics protocol

The amplicons obtained from each sample underwent purification with Ampure beads to eliminate any residual primers. Subsequently, an additional eight cycles of PCR were conducted, incorporating Illumina barcoded adapters to facilitate the preparation of sequencing libraries. The resulting libraries were purified using Ampure beads and quantified using a Qubit dsDNA High Sensitivity assay kit from Invitrogen (California, USA). The sequencing of DNA was carried out utilizing the Illumina Miseq platform with a 2x300PE v3 sequencing kit sourced from Illumina (Portland, USA).

The binary base call (BCL) data from the sequencer underwent demultiplexing to generate fastq raw data. The quality of the demultiplexed data was assessed through Fastqc (Version 0.11.9) and Multiqc (Version 1.10.1) tools. Samples that passed the quality control (QC) criteria were deemed eligible for subsequent analysis. Our metagenomics pipeline, designed explicitly for 16S and ITS metagenomics (Biokart Pipeline), was then applied to the qualified samples. After completing the run, the ultimate raw Operational Taxonomic Unit (OTU) table was obtained, serving as the foundation for the subsequent analysis visualisation. The abundance feature tables, depicting the presence of organisms in each sample, were crafted using Microsoft Excel (2021). Additional analyses, such as Heatmap, core microbiome, Dendrogram, Alpha diversity, Beta diversity, PCOA plot, and Rarefaction curve, were conducted utilizing Microbiomeanalyst, an online tool available at [https://www.microbiomeanalyst.ca/](https://www.microbiomeanalyst.ca/).

This workflow facilitates precise examinations at the genus level, ensuring high accuracy. Analysis of microbial diversity in various bio-composts was conducted using Alpha and Beta diversity indices. The utilized databases included SILVA, GREENGENES, and NCBI. Each read was classified based on...
percentage (%) coverage and identity. The 16S and ITS workflow prove valuable in comprehending microbial community composition and discerning beneficial and pathogenic microorganisms within a mixed sample [25, 26].

### Statistical analysis

To conduct advanced metagenomic data analysis, we categorized the samples into three groups, allowing for the exploration of alpha and beta diversity and the statistical significance of microbiome levels among different compost samples. Group 1 comprised pre-plantation floodplain soil samples: YC, YD, YK, YM, YF, and YF. Group 4 included post-harvest floodplain soil samples, YCRAH, YDRAH, YKRAH, YMRAH, YVRAH, and YFRAH. Group 6 encompassed post-harvest residential soil samples: NCRAH, NDRAH, NKRAH, and NFRAH. The data input for these sample groups underwent filtration, and alpha diversity was assessed using Chao1, Shannon-Weiner, Simpson, and Fisher, using the T-test and ANOVA statistical methods [27]. The beta diversity was constructed at the Genus taxonomic level with Bray-Curti’s index distance method based on the Permutational MANOVA (PERMANOVA) statistical method.

### Raw data deposition

The raw sequencing data generated from the Illumina MiSeq platform for the analysis of 16S and ITS metagenomic regions has been securely archived and can be accessed through the Indian Nucleotide Data Archive (INDA) of the Indian Biological Data Centre. The corresponding INDA Accession Number for the 16S metagenomic dataset is INRP000065, while the Accession Number INRP000089 identifies the ITS metagenomic dataset.

Furthermore, by international data standards, the 16S metagenomic study has been assigned the Bio-project Accession Number PRJEB62447 within the International Nucleotide Sequence Database Collaboration (INSDC). Likewise, the ITS metagenomic study has been assigned the Bio-project Accession Number PRJEB67873. These accession numbers are essential for proper documentation and retrieval of the data in the context of scientific research and data sharing (Table 1).

### Results

#### The physicochemical parameters of the soil

The pH levels of both pre-plantation and post-harvest soil samples exhibited moderate alkalinity, ranging from 7.66 to 8.56. The lowest pH was recorded in the fertilizer-amended soil (YF) at 7.66, while the highest pH was observed in cow dung manure-amended soil (YC) at 8.56. Electrical conductivity (EC) values varied, indicating non-saline to moderately saline conditions. The lowest EC was in municipal organic waste compost-amended soil (YMRAH) at 0.363 dS/m, whereas the highest was in vermicompost-amended soil (YV) at 0.938 dS/m. Other samples fell within the slightly saline category, with EC levels ranging from 0.44 dS/m to 0.775 dS/m.
Total organic carbon (TOC) content in pre-plantation floodplain soil ranged from 2.3–6.7%. The lowest TOC level was in chemical fertilizer-amended soil (YF), while the highest was in leaf compost-amended soil (YD). Kitchen waste compost (YK), cow dung manure (YC), and municipal organic waste compost (YM) amendments also exhibited high TOC levels at 6.6%, 6.6%, and 6.4%, respectively. Post-harvest soil samples had even higher TOC levels, ranging from 6.01–7.87%. In post-harvest floodplain soils, the lowest TOC was in the sample amended with chemical fertilizer (YFRAH). At the same time, cow dung manure (YCRAH) and leaf waste compost (YDRAH) amendments had the highest TOC levels at 7.87% and 7.72%, respectively. Among residential post-harvest soil samples, leaf (NDRAH) and kitchen waste compost (NKRAH) amendments exhibited higher TOC levels at 4.5% and 3.84%, respectively.

Total nitrogen levels in pre-plantation floodplain soil ranged from 0.10–1.41%. The lowest total Nitrogen was found in the fertilizer-amended soil (YF), while the highest was in vermicompost-amended soil (YV). In post-harvest floodplain soils, total Nitrogen ranged from 0.11–0.2%, with higher levels in vermicompost-amended soil (YVRAH) and cow dung manure-amended soil (YCRAH) at 0.2% and 0.19%, respectively. Among residential post-harvest soil samples, cow dung manure (NCRAH) and kitchen waste compost (NKRAH) amendments had higher total nitrogen levels at 1.35% and 0.82%, respectively (Table 2).
Table 2
The physicochemical parameters of the soil samples

<table>
<thead>
<tr>
<th>Samples of different soil types</th>
<th>pH</th>
<th>Electrical Conductivity (EC) (dS/m)</th>
<th>Total carbon (%)</th>
<th>Total Nitrogen (%)</th>
<th>Sulphur (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>YC</td>
<td>8.56 ± 0.43</td>
<td>0.607 ± 0.030</td>
<td>6.6 ± 0.3</td>
<td>0.12 ± 0.0</td>
<td>4.7 ± 0.2</td>
</tr>
<tr>
<td>YD</td>
<td>8.18 ± 0.41</td>
<td>0.605 ± 0.030</td>
<td>6.7 ± 0.3</td>
<td>0.17 ± 0.01</td>
<td>1.7 ± 0.1</td>
</tr>
<tr>
<td>YK</td>
<td>7.8 ± 0.39</td>
<td>0.755 ± 0.038</td>
<td>6.6 ± 0.3</td>
<td>0.70 ± 0.01</td>
<td>0.1 ± 0</td>
</tr>
<tr>
<td>YM</td>
<td>8.03 ± 0.40</td>
<td>0.775 ± 0.039</td>
<td>6.4 ± 0.3</td>
<td>0.22 ± 0.01</td>
<td>5.2 ± 0.3</td>
</tr>
<tr>
<td>YV</td>
<td>7.7 ± 0.38</td>
<td>0.938 ± 0.047</td>
<td>3.7 ± 0.2</td>
<td>1.41 ± 0.1</td>
<td>0.2 ± 0.0</td>
</tr>
<tr>
<td>YF</td>
<td>7.66 ± 0.38</td>
<td>0.64 ± 0.032</td>
<td>2.3 ± 0.1</td>
<td>0.10 ± 0.0</td>
<td>0.8 ± 0.0</td>
</tr>
<tr>
<td>YCRAH</td>
<td>8.16 ± 0.41</td>
<td>0.44 ± 0.022</td>
<td>7.87 ± 0.4</td>
<td>0.19 ± 0.01</td>
<td>0.25 ± 0</td>
</tr>
<tr>
<td>YDRAH</td>
<td>8.12 ± 0.40</td>
<td>0.467 ± 0.023</td>
<td>7.72 ± 0.4</td>
<td>0.17 ± 0.01</td>
<td>0.2 ± 0</td>
</tr>
<tr>
<td>YKRAH</td>
<td>8.03 ± 0.41</td>
<td>0.51 ± 0.026</td>
<td>6.36 ± 0.03</td>
<td>0.16 ± 0.01</td>
<td>0.28 ± 0</td>
</tr>
<tr>
<td>YMRAH</td>
<td>8.46 ± 0.42</td>
<td>0.363 ± 0.018</td>
<td>7.33 ± 0.4</td>
<td>0.11 ± 0.1</td>
<td>0.29 ± 0</td>
</tr>
<tr>
<td>YVRAH</td>
<td>8.19 ± 0.41</td>
<td>0.463 ± 0.023</td>
<td>7.42 ± 0.4</td>
<td>0.2 ± 0.01</td>
<td>0.27 ± 0</td>
</tr>
<tr>
<td>YFRAH</td>
<td>8.01 ± 0.40</td>
<td>0.442 ± 0.022</td>
<td>6.01 ± 0.3</td>
<td>0.15 ± 0.01</td>
<td>0.26 ± 0</td>
</tr>
<tr>
<td>NCRAH</td>
<td>8.23 ± 0.41</td>
<td>0.539 ± 0.027</td>
<td>2.16 ± 0.11</td>
<td>1.35 ± 0.07</td>
<td>0.04 ± 0</td>
</tr>
<tr>
<td>NDRAH</td>
<td>8.37 ± 0.42</td>
<td>0.593 ± 0.029</td>
<td>4 ± 0.2</td>
<td>0.52 ± 0.03</td>
<td>0.04 ± 0</td>
</tr>
<tr>
<td>NKRAH</td>
<td>8.23 ± 0.41</td>
<td>0.535 ± 0.027</td>
<td>3.84 ± 0.19</td>
<td>0.82 ± 0.04</td>
<td>0.05 ± 0</td>
</tr>
<tr>
<td>NFRAH</td>
<td>8.24 ± 0.41</td>
<td>0.617 ± 0.031</td>
<td>2.52 ± 0.13</td>
<td>0.74 ± 0.04</td>
<td>0.04 ± 0</td>
</tr>
</tbody>
</table>

This table provides a comprehensive overview of the physicochemical parameters of different soil samples. All samples displayed a moderately alkaline pH ranging from 7.66 to 8.56. The electrical conductivity (EC) before plantation ranged from 0.64 to 0.938 mS/cm, with a slight reduction post-
harvest to a range of 0.363 to 0.617 mS/cm. All the values represent the mean of triplicate measurements (N = 3).

**Microbiome richness and diversity of the soils amended with organic composts**

The 16S and ITS metagenomic analyses revealed substantial variations in library sizes among the soil samples. Vermicompost-amended post-harvest soil (YVRAH) exhibited the smallest 16S metagenomic library size (38,292 reads). In comparison, pre-plantation soil amended with chemical fertilizer (YF) had the most extensive library size (335,750 reads). Except for leaf compost-amended soil samples (YD and YDRAH), there was a reduction in the library size of 16S metagenomics in all post-harvest soil samples (Supplementary Fig. S1).

The ITS metagenomic profiling also displayed significant variations in library size, ranging from 14 to 102,569. Cow dung-amended post-harvest soil had the lowest library size, whereas kitchen compost-amended pre-plantation soil (YK) had the highest. Post-harvest soils amended with cow dung, municipal organic waste compost, and kitchen waste compost exhibited reduced ITS library sizes. In contrast, those amended with vermicompost, leaf compost, and chemical fertilizer showed increased sizes.

Vermicompost-amended soils (YV and YVRAH) had the most significant increment in ITS library size, going from 25,594 to 78,023.

All 16S and ITS metagenomic profiling samples underwent rarefaction to achieve an even sequencing depth, determined by the sample with the lowest sequencing depth. The analysis was then visualized using the filtered data source. The results displayed rarefaction curves for 16S and ITS, indicating that all sequencing reads were thoroughly sampled. Interestingly, the rarefaction curve for 16S profiling revealed higher completeness in pre-plantation soils than in post-harvest soil samples. Conversely, the rarefaction curve for ITS metagenomic profiling indicated higher completeness in post-harvest soil samples than in pre-plantation soils (Fig. 1).

The Alpha diversity in 16S and ITS metagenomic profiles was assessed using Chao1, Shannon, Simpson, and Fisher, with statistical analysis performed using T-tests and ANOVA. The alpha diversity indices exhibited variations among soil samples collected before and after harvest, which had been amended with various types of compost. Generally, the pre-plantation soil samples demonstrated higher alpha diversity indices in 16S metagenomics than post-harvest soil samples. Among the pre-plantation soils, those amended with chemical fertilizer and vermicompost displayed significantly higher alpha diversity indices than other soil samples. In contrast, those amended with cow dung manure exhibited lower indices.

In contrast, the post-harvest soils, mainly the sample amended with kitchen waste compost, exhibited the highest alpha diversity indices in 16S metagenomics, while samples amended with leaf compost and chemical fertilizer displayed comparatively lower alpha diversity indices (Table 3). Additionally, it is worth noting that the floodplain soil samples in the post-harvest category displayed higher alpha diversity
indices than the residential soils. The p-values for the 16S metagenomic alpha diversity indices (Chao1, Fisher, Shannon, and Simpson) were determined to be 0.0079, 0.00014, 0.0057, and 0.11, respectively, with ANOVA F-values of 7.17, 18.95, 7.90, and 2.66 (Fig. 2). The leaf compost and cow dung manure amended soils had a higher alpha diversity index of the ITS metagenomic profiling among the pre-plantation soil samples. Conversely, the soil sample amended with municipal organic waste compost demonstrated the lowest alpha diversity among the pre-plantation soils. Notably, alpha diversity indices were increased in post-harvest soils amended with leaf litter and kitchen waste compost

<table>
<thead>
<tr>
<th>Samples</th>
<th>Chao1 index</th>
<th>Fisher Index</th>
<th>Shannon Index</th>
<th>Simpson Index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>16S</td>
<td>ITS</td>
<td>16S</td>
<td>ITS</td>
</tr>
<tr>
<td>YC</td>
<td>189.8 ± 5.5</td>
<td>10 ± 4.11</td>
<td>34.60</td>
<td>5.57</td>
</tr>
<tr>
<td>YD</td>
<td>192.5 ± 5.7</td>
<td>10 ± 4.11</td>
<td>35.31</td>
<td>5.57</td>
</tr>
<tr>
<td>YK</td>
<td>195.0 ± 5.9</td>
<td>12 ± 7.94</td>
<td>35.79</td>
<td>3.98</td>
</tr>
<tr>
<td>YM</td>
<td>203.5 ± 6.8</td>
<td>7 ± 4.34</td>
<td>37.23</td>
<td>1.87</td>
</tr>
<tr>
<td>YV</td>
<td>200.7 ± 4.0</td>
<td>9 ± 4.10</td>
<td>38.20</td>
<td>3.98</td>
</tr>
<tr>
<td>YF</td>
<td>219.0 ± 15.3</td>
<td>12 ± 7.05</td>
<td>36.03</td>
<td>3.98</td>
</tr>
<tr>
<td>YCRAH</td>
<td>179.4 ± 3.6</td>
<td>10 ± 4.11</td>
<td>33.41</td>
<td>5.57</td>
</tr>
<tr>
<td>YDRAH</td>
<td>176.4 ± 7.2</td>
<td>15.5 ± 8.07</td>
<td>30.84</td>
<td>7.76</td>
</tr>
<tr>
<td>YKRAH</td>
<td>191.1 ± 7.8</td>
<td>16.5 ± 8.08</td>
<td>33.88</td>
<td>10.88</td>
</tr>
<tr>
<td>YMRAH</td>
<td>186.6 ± 6.8</td>
<td>5.5 ± 1.25</td>
<td>33.18</td>
<td>2.78</td>
</tr>
<tr>
<td>YVRAH</td>
<td>182.7 ± 4.0</td>
<td>7.25 ± 0.73</td>
<td>33.88</td>
<td>5.57</td>
</tr>
<tr>
<td>YFRAH</td>
<td>177.1 ± 6.2</td>
<td>6.5 ± 1.27</td>
<td>31.54</td>
<td>3.98</td>
</tr>
<tr>
<td>NCRAH</td>
<td>162.9 ± 4.5</td>
<td>3 ± 0.41</td>
<td>29.01</td>
<td>1.17</td>
</tr>
<tr>
<td>NDRAH</td>
<td>166.2 ± 6.7</td>
<td>7.5 ± 2.54</td>
<td>28.78</td>
<td>3.98</td>
</tr>
<tr>
<td>NKRAH</td>
<td>199.8 ± 11.5</td>
<td>9 ± 4.10</td>
<td>33.41</td>
<td>3.98</td>
</tr>
<tr>
<td>NFRAH</td>
<td>171.4 ± 9.9</td>
<td>6.5 ± 1.27</td>
<td>28.32</td>
<td>1.17</td>
</tr>
</tbody>
</table>

This table shows the alpha diversity indices of different soil samples. The microbial diversity was relatively higher in the pre-plantation soils than post-harvest soils. The chemical fertilizer, municipal organic waste compost, and vermicompost-amended soils had higher levels of microbial diversity before plantation. The leaf litter and kitchen waste compost amended soil had higher levels of microbial diversity in the post-harvest soils.
The post-harvest soil samples amended with leaf litter, kitchen waste compost and vermicompost had a higher alpha diversity index than the other samples. The statistical p-values for the Chao1, Fisher, Shannon, and Simpson alpha diversity indices in ITS metagenomic profiling were determined to be 0.14344, 0.065365, 0.031878, and 0.046761, respectively, with ANOVA F-values of 2.2631, 3.3894, 4.5445, and 3.9123. These results provide statistical evidence of the differences in alpha diversity among the various soil samples and their respective compost amendments in the study.

Beta diversity analysis was conducted at the genus taxonomic level employing the Bray-Curtis index distance method for the 16S and ITS metagenomic analyses. These analyses were statistically evaluated using the Permutational MANOVA (PERMANOVA) method. The results yielded a notable statistical significance, as indicated by p-values of less than 0.01 for the 16S and ITS metagenomic datasets. Specifically, the PERMANOVA F-values were determined to be 9.2961 for the 16S analysis and 3.6166 for the ITS analysis. This analysis unequivocally established the presence of significant dissimilarities among the sample groups regarding their microbiome diversity (Fig. 3).

### Taxonomic classification of the bacterial and fungal communities in the soils

#### At the phylum level

The OTU table provides insights into the microbial community composition in soil samples, revealing around twenty bacterial phyla and eight fungal phyla. Taxonomic classifications extend across various hierarchical levels, including Classes, Orders, Families, Genera, and Species. Key bacterial phyla observed in all soil samples encompassed *Proteobacteria, Planctomycetes, Actinobacteria, Firmicutes, Chloroflexi, Acidobacteria, Bacteroidetes, Verrucomicrobia, Gemmatimonadetes, Armatimonadetes, Cyanobacteria, Euryarchaeota, Nitrospirae,* and *Chlamydiae.* Dynamic shifts in these phyla, influenced by diverse compost amendments, were evident between pre-plantation and post-harvest soil samples (Supplementary Fig. S2).

The types and levels of bacterial and fungal phyla identified varied based on the nature of the bio-compost used, showcasing differences between pre-plantation and post-harvest soils. *Firmicutes* were highest in fertilizer-amended pre-plantation soil but decreased significantly in post-harvest soil. In contrast, leaf and kitchen waste compost-amended post-harvest soil experienced an increase. *Chloroflexi* and *Bacteroidetes* counts rose in post-harvest soil amended with leaf waste compost but decreased in fertilizer and other bio-compost-amended soils. *Acidobacteria, Armatimonadetes,* and *Nitrospirae* increased in post-harvest soil, contrasting with decreases in soils amended with chemical fertilizers, municipal organic waste compost, and vermicompost. *Gemmatimonadetes* increased in all post-harvest soil samples, while *Cyanobacteria, Euryarchaeota,* and *Thermi* decreased (Supplementary Table S1).

Proteobacteria abundance declined significantly in post-harvest soils, except in leaf waste compost-amended soil, where a notable increase of 98.30% occurred. The most substantial reduction (66.87%)
was observed in vermicompost-amended soil, followed by a 49.34% decrease in fertilizer-amended soil. Planctomycetes increased in post-harvest soils amended with cow dung manure, leaf waste compost, and kitchen waste compost, with the highest increase (218%) in the latter. Actinobacteria counts increased in post-harvest soil samples amended with cow dung manure, leaf waste compost, and kitchen waste compost, with the most significant increase (226%) in the former.

Abundant fungal phyla across all soil samples were consistently Ascomycota and Basidiomycota. Other fungal phyla, including Mucoromycota, Olpidiomycota, and Zoopagomycota, were detected at notably elevated levels (Supplementary Table S2). In pre-plantation soils, the highest counts of Ascomycota were recorded in the sample amended with leaf and kitchen waste compost. In contrast, municipal organic waste compost and vermicompost-amended samples exhibited the lowest counts. In post-harvest soils, chemical fertilizer and vermicompost-amended soils displayed the highest Ascomycota counts. Basidiomycota's pre-plantation soil sample amended with kitchen waste compost recorded the highest count. In contrast, the post-harvest soil sample amended with leaf waste compost exhibited the highest Basidiomycota count. In contrast, the soil amended with cow dung consistently demonstrated the lowest Basidiomycota count in both pre-plantation and post-harvest soil samples.

Post-harvest soil samples exhibited a notable decline in overall Operational Taxonomic Units (OTUs) within the fungal microbiome following the addition of cow dung manure, kitchen waste compost, and municipal organic waste compost. In contrast, introducing leaf litter compost, vermicompost, and chemical fertilizer resulted in a marked augmentation of the total OTUs within the fungal microbiome. Vermicompost demonstrated the most substantial increase in overall OTU richness within the fungal microbiome.

**At the genus level**

The dynamics of the bacterial genera in the pre-plantation and post-harvest soil samples varied depending on the type of amendments used, such as chemical fertilizer and different types of bio-composts. About thirty bacterial genera constitute the core microbiome, detected above a threshold level across the samples. The heat mapping showed that the levels of these core microbiomes varied in different soil samples depending on the types of composts used for soil amendment (Fig. 5).

Several beneficial genera with higher Operational Taxonomic Units (OTUs) were consistently identified across the soil samples. These included Agromyces, Bacillus, Flavobacterium, Nitrospira, Planctomyces, Pseudomonas, Streptomyces, Myxococcus, Rhodoplanes, Alicyclobacillus, and Steroidobacter. Counts of these genera varied between pre-plantation and post-harvest soils and among different compost amendments (Supplementary Table S3).

The counts of Agromyces were lower in the post-harvest soil samples than in pre-plantation soils. The chemical fertilizer-amended soil had the highest counts of Agromyces among the pre-plantation soils. However, the leaf compost-amended soil had the highest count among the post-harvest soil samples. The Bacillus levels were increased in the post-harvest soil samples amended with cow dung manure, leaf
waste compost, and kitchen waste compost. The leaf waste compost amended soil sample showed the most extraordinary increment of about 99%. Among the post-harvest soil samples, the kitchen waste compost amended soil sample had the highest level of *Bacillus* in both the floodplain and residential soil. *Flavobacterium* counts were higher in the pre-plantation soils than in the post-harvest soils in all the samples. The fertilizer-amended soil samples had the highest counts of the *Flavobacterium* in both the pre-plantation and post-harvest soil samples (Fig. 6).

Meanwhile, the cow dung-amended soils had the lowest counts of the genus in all the samples. *Nitrospira* was identified with the highest count in fertilizer-amended soil, followed by municipal organic waste compost-amended soil among the pre-plantation soil samples. However, its count was reduced to half in the post-harvest soil samples. There was an increase in the *Nitrospira* counts in the post-harvest soil samples amended with cow dung manure, leaf waste compost, and kitchen waste compost. The count of *Nitrospira* was relatively higher in the fertilizer, cow dung manure and leaf waste compost-amended soil samples compared to municipal organic waste compost and vermicompost-amended soil samples amongst the post-harvest soil.

*Planctomyces* counts were relatively high in all the soil samples. The highest counts were seen in the fertilizer and municipal organic waste compost-amended soil in the pre-plantation soil samples. Nonetheless, the post-harvest soil samples reduced its count to almost half. At the same time, there was an increase in the counts of *Planctomyces* in the post-harvest soils amended with cow dung manure, leaf waste compost, and kitchen waste compost. The leaf waste compost-amended soil sample had the highest level of *Planctomycetes* among the post-harvest soil samples. *Pseudomonas* was higher in the pre-plantation soil samples than the post-harvest soil samples. In the pre-plantation soil samples, the kitchen waste compost and chemical fertilizer-amended soils had relatively higher counts of *Pseudomonas*. However, the post-harvest soils had relatively similar levels of this genus, though slightly higher in the chemical fertilizer-amended sample. *Streptomyces* were observed with a relatively high count in the pre-plantation soils, but their count was reduced drastically in the post-harvest soil samples. The pre-plantation soil samples amended with municipal organic waste compost, chemical fertilizer, and cow dung manure were seen with relatively higher *Streptomyces* among the pre-plantation soil samples. *Steroidobacter* was identified with relatively higher counts in the post-harvest soil samples than in the pre-plantation soils. The municipal organic waste compost-amended soil had the highest level in the pre-plantation soil samples. Meanwhile, the *Steroidobacter* counts became highest in the leaf and kitchen waste compost-amended soil samples among the post-harvest soil samples (*Supplementary Table S4*).

The prevalent pathogenic bacterial genera identified within the collected samples included *Agrobacterium, Corynebacterium, Burkholderia, Prevotella, Legionella*, and *Nocardia*. In the pre-plantation soil samples, *Agrobacterium* was present at negligible levels, except in the leaf waste compost-amended soil, where its presence was more pronounced. Conversely, the post-harvest soil samples exhibited an overall increase in *Agrobacterium* counts. Notably, the municipal organic waste compost amendment yielded the highest *Agrobacterium* counts among the post-harvest soil samples. *Conversely, Corynebacterium* exhibited relatively higher counts in the kitchen compost-amended soil compared to
other amendments, both in the pre-and post-harvest soils. In contrast, *Legionella* counts were relatively higher in the chemical fertilizer-amended soil than in the compost-amended soils in both pre-plantation and post-harvest samples. Furthermore, the chemical fertilizer-amended post-harvest soil displayed higher counts of *Burkholderia* and *Nocardia* than the compost-amended soils (Supplementary Table S5 & S6).

A comprehensive analysis of the soil samples has revealed the presence of thirty-three fungal genera that constitute the core fungal microbiome consistently across all samples. Notably, the abundance of these genera exhibited considerable variability between the pre-plantation and post-harvest soil samples and across distinct compost amendments. Among the identified fungal genera, several stand out for their potential beneficial influence on soil fertility and plant health. These noteworthy genera encompass *Wallemia*, *Ascobolus*, *Aspergillus*, *Pichia*, *Microascus*, *Cephalotrichum*, *Coprinus*, *Mortierella*, *Arthrographis*, and *Trichoderma* (Fig. 7).

These fungal taxa are particularly interesting due to their ability to impact the soil ecosystem and promote plant health positively. The quantification of *Wallemia* populations exhibited consistent prominence across all soil samples. Notably, the pre-plantation soil subjected to kitchen compost amendment displayed the highest *Wallemia* abundance, whereas the post-harvest soil samples following leaf compost amendment exhibited the most substantial *Wallemia* counts. It is pertinent to highlight that, overall, there was a reduction in the *Wallemia* population in post-harvest soils compared to their pre-plantation counterparts. However, a notable exception to this trend was observed in the leaf and vermicompost amendments, where an increase in *Wallemia* counts was evident (Fig. 8).

The genera such as *Ascobolus*, *Aspergillus*, and *Microascus* were identified at relatively high levels within the soil samples. Notably, the counts of *Ascobolus* were observed to be higher in the post-harvest soil samples compared to the pre-plantation soils, with the highest counts documented in the leaf compost and chemical fertilizer amendments. Conversely, the counts of *Aspergillus* and *Microascus* exhibited a decrease in the post-harvest soils as opposed to their pre-plantation counterparts. Pre-plantation soil samples showed the highest levels of *Aspergillus* and *Microascus* in the leaf compost amendment. However, in post-harvest soils, the counts of *Aspergillus* reached their zenith in the chemical fertilizer amendment, while the counts of *Microascus* were most pronounced in the vermicompost amendment. In distinction, *Pichia* was abundant exclusively in the leaf compost amendment within the post-harvest soils (Supplementary Table S7 & S8).

The prominent pathogenic fungal genera detected with a relative count include *Fusarium*, *Olpidium*, *Penicillium*, *Acremonium*, *Alternaria*, *Cladosporium*, *Rhizoctonia*, *Verticillium*, and *Scopulariopsis*. The levels of these pathogenic genera were reduced in the post-harvest soil samples, except in the vermicompost amendment in which there was an increase in the level of *Fusarium*, *Olpidium*, *Cladosporium*, *Rhizoctonia* and *Verticillium*. Interestingly, the counts of these pathogenic genera were utterly absent in the post-harvest soil amended with cow dung manure. It is worth noting that, generally,
the floodplain soil samples exhibited relatively higher levels of pathogenic genera in comparison to the residential soil samples within the post-harvest soil dataset (Supplementary Table S9 & S10).
Table 4
Dynamics of the beneficial bacterial and fungal genera in pre-plantation and post-harvest soils

<table>
<thead>
<tr>
<th>16S Metagenomics</th>
<th>Beneficial Bacterial Genera</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil sample of compost amendment</td>
<td>Sum OTUs in Pre-plantation soils</td>
</tr>
<tr>
<td>Cow dung amendment</td>
<td>8618</td>
</tr>
<tr>
<td>Leaf compost amendment</td>
<td>6047</td>
</tr>
<tr>
<td>Kitchen compost amendment</td>
<td>12786</td>
</tr>
<tr>
<td>Municipal compost amendment</td>
<td>20304</td>
</tr>
<tr>
<td>Vermicompost amendment</td>
<td>7146</td>
</tr>
<tr>
<td>Chemical fertilizer amendment</td>
<td>24423</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>ITS Metagenomics</th>
<th>Beneficial Fungal Genera</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil sample of compost amendment</td>
<td>Sum OTUs in Pre-plantation soils</td>
</tr>
<tr>
<td>Cow dung amendment</td>
<td>34363</td>
</tr>
<tr>
<td>Leaf compost amendment</td>
<td>47813</td>
</tr>
<tr>
<td>Kitchen compost amendment</td>
<td>78944</td>
</tr>
<tr>
<td>Municipal compost amendment</td>
<td>30490</td>
</tr>
<tr>
<td>Vermicompost amendment</td>
<td>21641</td>
</tr>
<tr>
<td>Chemical fertilizer amendment</td>
<td>59865</td>
</tr>
</tbody>
</table>
This table shows the dynamic of the beneficial bacterial and fungal genera in the pre-plantation and post-harvest soils. The leaf compost amendment showed an increase in the beneficial bacterial genera in the post-harvest soil, while there was a reduction in all the other amendments. The beneficial fungal genera showed an increase in the leaf compost and vermicompost amendments, while other amendments showed a reduction in the post-harvest soils.

**Bio-fertilizing potential of different compost amendments**

We explore the influence of various compost amendments on soil microbial composition before planting and after harvesting, revealing significant effects on beneficial and pathogenic microbiome structures. Following the harvest, there was a notable decrease in the total Operational Taxonomic Units (OTUs) linked to beneficial bacterial genera in all compost-amended soils, except leaf waste compost, demonstrating a remarkable 116% increase. The soil amended with chemical fertilizer, initially boasting the highest count of beneficial genera, experienced a significant 49% reduction post-harvest. Similar reductions were observed in soils amended with cow dung manure, kitchen waste compost, municipal organic waste compost, and vermicompost, with the most substantial reduction occurring in municipal organic waste compost (54%) and the least in kitchen waste compost (12%). Cow dung manure had the highest proportion (57%) of beneficial bacterial OTUs relative to the total OTU count, while vermicompost had the lowest (39%). Conversely, leaf waste compost had the highest ratio (57%) of beneficial bacterial genera, and kitchen waste compost had the lowest (46%) post-harvest (Table 4).
Table 5
Dynamics of the pathogenic genera in pre-plantation and post-harvest soils

<table>
<thead>
<tr>
<th>16S Metagenomics</th>
<th>Pathogenic Genera</th>
<th>Compost-amended soil samples</th>
<th>Sum OTUs in Pre-plantation soils</th>
<th>Ratio to the total OTUs</th>
<th>Sum OTUs in post-harvest soils</th>
<th>Ratio to the total OTUs</th>
<th>% Change from pre- to post-harvest soils</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cow dung amendment</td>
<td>45</td>
<td>0.29%</td>
<td>51</td>
<td>0.64%</td>
<td>13%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leaf compost amendment</td>
<td>588</td>
<td>4.79%</td>
<td>241</td>
<td>1.04%</td>
<td>-59%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kitchen compost amendment</td>
<td>477</td>
<td>1.49%</td>
<td>171</td>
<td>0.69%</td>
<td>-64%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Municipal compost amendment</td>
<td>232</td>
<td>0.55%</td>
<td>238</td>
<td>1.20%</td>
<td>3%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vermicompost amendment</td>
<td>119</td>
<td>0.65%</td>
<td>57</td>
<td>0.89%</td>
<td>-52%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chemical fertilizer amendment</td>
<td>98</td>
<td>0.20%</td>
<td>227</td>
<td>0.94%</td>
<td>132%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ITS Metagenomics</td>
<td>Pathogenic Genera</td>
<td>Compost-amended soil samples</td>
<td>Sum OTUs in Pre-plantation soils</td>
<td>Ratio to the total OTUs</td>
<td>Sum OTUs in post-harvest soils</td>
<td>Ratio to the total OTUs</td>
<td>% Change from pre- to post-harvest soils</td>
</tr>
<tr>
<td>Cow dung amendment</td>
<td>8722</td>
<td>18%</td>
<td>2</td>
<td>13%</td>
<td>-100%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leaf compost amendment</td>
<td>10209</td>
<td>14%</td>
<td>4071</td>
<td>5%</td>
<td>-60%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kitchen compost amendment</td>
<td>18276</td>
<td>17%</td>
<td>3668</td>
<td>10%</td>
<td>-80%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Municipal compost amendment</td>
<td>3403</td>
<td>9%</td>
<td>2080</td>
<td>6%</td>
<td>-39%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vermicompost amendment</td>
<td>2786</td>
<td>10%</td>
<td>5359</td>
<td>6%</td>
<td>92%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chemical fertilizer amendment</td>
<td>14398</td>
<td>17%</td>
<td>3516</td>
<td>4%</td>
<td>-76%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The diversity of beneficial fungal microbiome Operational Taxonomic Units (OTUs) varied across amendments, with kitchen waste compost having the highest count before planting and vermicompost having the highest post-harvest. Reductions in beneficial fungal OTUs were observed in soils amended with cow dung manure, kitchen waste compost, municipal organic waste compost, and chemical fertilizer. At the same time, vermicompost exhibited the most substantial increase (196%), and leaf waste compost showed a 21% increase.

The dynamics of pathogenic bacterial and fungal microbiomes also varied with compost amendments. Post-harvest, leaf, kitchen, and vermicompost-amended soils exhibited reduced pathogenic bacterial OTUs, with kitchen compost showing the most substantial reduction (64%). Conversely, chemical fertilizer, cow dung, and municipal organic waste compost amendments led to increased pathogenic bacterial OTUs, with the most significant increase in chemical fertilizer (132%). Among the pre-plantation soil samples, pathogenic fungal Operational Taxonomic Units (OTUs) were more abundant in soils amended with kitchen waste compost and fertilizer. Following the harvest decreases in pathogenic fungal OTUs were noted in most compost-amended soils, except for vermicompost, which experienced a 92% increase. Cow dung manure and kitchen waste compost showed notable reductions of approximately 80% and 60%, respectively, while municipal organic waste compost exhibited the least reduction (39%) (Table 5).

Discussion

The effect of the bio-compost amendment on the microbial richness and diversity in the soils

The investigation into the impact of bio-compost amendments on soil microbial richness and diversity unveils the intricate relationship between various factors and the composition of bacterial and fungal microbiomes. Microbial diversity, often linked to heightened soil ecosystem functioning and resilience, is shaped by soil type, agricultural practices, amendments, and environmental conditions [28, 29]. Heightened microbial diversity is generally associated with heightened soil ecosystem functioning and resilience. The variability in the total number of Operational Taxonomic Units (OTUs) derived from 16S and Internal Transcribed Spacer (ITS) metagenomic analyses across distinct soil samples signifies differences in the bacterial and fungal microbiome structure within the soil samples. These variations can be attributed to distinctions in soil characteristics, the nature of bio-compost amendments, and interactions between compost and soil microbes within the cropping system. The variation in Operational Taxonomic Units (OTUs) across distinct soil samples, as derived from 16S and Internal Transcribed Spacer (ITS) metagenomic analyses, indicates differences in microbiome structure influenced by soil characteristics, bio-compost nature, and interactions within the soil ecosystem [30, 31].

Post-harvest soils generally reduce total OTUs compared to pre-plantation soils, except leaf waste compost-amended soils, where an increase in total OTUs is observed in the 16S metagenomic analysis. This reduction aligns with previous findings indicating that disturbances, such as harvesting, can disrupt
microbial communities and reduce overall diversity. However, the observed increase in OTUs in leaf waste compost-amended soil suggests favourable conditions for microbial growth fostered by this specific bio-compost. Notably, the ITS metagenomic analysis reveals a significant increase in total OTUs within the fungal microbiome of post-harvest soils amended with leaf and vermicompost, emphasizing their capacity to promote fungal growth. Compost amendments, known to enhance soil fertility and microbial activity, contribute to increased microbial diversity [32].

It is essential to recognize that bio-composts from various sources may differ in quality, influenced by nutrient content and potentially toxic elements [18]. The impact of bio-compost amendments on soil microbial community composition remains an essential area of research. Some studies have explored how microbial community composition changes following bio-compost amendments. For instance, recent research employing high-throughput sequencing techniques found that long-term bio-compost application significantly altered the microbial community structure, resulting in an increased abundance of beneficial microbial groups such as *Sphingomonas*, *Acidibacter*, and *Nocardioides* and a reduction in the relative abundance of pathogenic microorganisms like *Stachybotrys* [3]. Our findings align with studies showing that long-term bio-compost application significantly alters microbial community structure, favouring beneficial groups and reducing pathogenic microorganisms [33]. Bio-compost amendments, including leaf waste compost, kitchen waste compost, vermicompost, municipal organic waste compost, and cow dung manure, enhance microbial diversity, which is critical for soil health and ecosystem functioning [7].

Research on the functional potential of microbial communities in compost-amended soils reveals an enrichment of genes associated with organic matter decomposition, nutrient recycling, and plant-microbe interactions, suggesting a positive influence on soil microbial functions [34]. The presence of beneficial microorganisms in bio-composts enhances soil fertility, promotes plant health, and improves nutrient availability, as demonstrated by increased plant growth-promoting bacteria and mycorrhizal fungi [35]. The long-term effects of bio-compost amendments on soil microbial dynamics are also a subject of investigation, with evidence suggesting that the impact on microbial community composition and diversity persists several years after initial application, highlighting the potential long-term benefits of bio-compost amendments [4].

**Bio-fertilizing potential of compost amendments**

The bio-fertilizing potential of compost amendments significantly contributes to soil fertility and the growth of beneficial microorganisms. Compost, rich in bacteria, fungi, protozoa, and nematodes, actively participates in nutrient recycling, breaking down organic matter to release readily available plant nutrients. Incorporating compost enriches the soil with beneficial microorganisms, fostering microbial diversity, activity, and biomass [36]. This augmentation of microbial diversity, activity, and biomass in soil following compost amendments leads to several benefits for soil fertility, nutrient recycling, and plant growth. Moreover, it fosters the colonization of soil with diverse microbial communities, increasing functional diversity and soil ecosystem resilience [37]. Our investigation demonstrates that incorporating bio-composts, such as leaf waste compost, cow dung manure, kitchen waste compost, and municipal
organic waste compost, augmented the abundance of beneficial bacteria such as *Achromobacter*, *Agromyces*, *Clostridium*, *Nitrospira*, *Planctomyces*, *Pseudomonas*, *Steroidobacter*, and *Streptomyces*, among others. These additions also increased the presence of beneficial fungal genera, including *Wallemia*, *Ascobolus*, *Pichia*, *Microascus*, and *Coprinus*. These microorganisms have significant potential for enhancing soil and plant health through nutrient solubilization, nitrogen fixation, bio-control, and plant growth promotion [38]. The substantial increase in beneficial microorganisms observed in post-harvest soil amended with leaf waste compost suggests that this specific compost can be a viable bio-organic fertilizer for sustainable agricultural productivity. In contrast, the decrease in beneficial microorganisms observed in post-harvest soil amended with chemical fertilizers reinforces the notion that chemical inputs may adversely impact soil microbial ecosystems in the long run [39, 40].

Compost-amended soils benefit from improved nutrient availability and cycling, resulting in enhanced plant nutrient uptake and reduced risks of nutrient leaching. Furthermore, microorganisms derived from compost contribute to forming and stabilizing soil aggregates, improving soil structure. This, in turn, enhances soil porosity, water infiltration, and water-holding capacity, ultimately improving moisture retention in the root zone. Microorganisms in compost can improve soil's physical properties and mitigate the negative consequences of soil compaction [41]. Overall, the study underscores the importance of bio-compost amendments in fostering soil health, microbial diversity, and sustainable agricultural productivity.

**Biocontrol of pathogenic microorganisms in the soil with compost amendment**

Efforts to remediate pathogenic microorganisms in soil focus on leveraging natural processes, and bio-composts have gained recognition for their potential in bioremediation. Their diverse microbial communities contribute to soil health through microbial competition, antibiosis, predation, production of antimicrobial compounds, and alteration of environmental conditions. Bio-composts' efficacy in bioremediation varies based on factors like compost composition, application rates, and environmental conditions, necessitating consideration of site-specific factors in implementation. Composts can contribute to the bioremediation of pathogenic microorganisms in the soil through various mechanisms, including microbial competition, antibiosis, predation, the production of antimicrobial compounds, and the alteration of environmental conditions (e.g., pH or moisture) that are unfavourable for pathogen survival [42, 43].

In our study, we assessed the effectiveness of different bio-composts, such as leaf waste compost, kitchen waste compost, cow dung manure, vermicompost, and municipal organic waste compost, in comparison to chemical fertilizers, in the bioremediation of soil-borne pathogens. We observed that the number of bacterial pathogenic Operational Taxonomic Units (OTUs) was significantly reduced in post-harvest soils amended with leaf, kitchen, and vermicompost. Additionally, soil samples amended with chemical fertilizers exhibited a relatively higher level of pathogenic OTUs, both in pre-plantation and post-harvest soils, compared to soil samples amended with bio-composts. Adding bio-composts was
associated with a higher degree of bioremediation of pathogenic microorganisms in post-harvest soils, including genera like Flavobacterium, Prevotella, Leptolyngbya, and Nocardia. Recent studies have also demonstrated the efficacy of bio-composts in bioremediation of phytopathogens. For example, research has reported the disease-suppressive properties of thermophilic compost and vermicompost on a wide range of phytopathogens, including Rhizoctonia, Phytophthora, Plasmodiophora brassicae, Gaeumannomyces graminis, and Fusarium species. Bio-composts have also proven effective in controlling arthropods and nematodes, improving overall soil health [42, 44].

Moreover, researchers have explored the antagonistic activity of microorganisms isolated from bio-composts against soil-borne plant pathogens, finding them effective in suppressing pathogens such as Fusarium species [45][46]. Green waste compost and compost derivatives, including compost extract, compost tea, and the solid phase after extraction, have also demonstrated strong inhibitory effects on the growth of plant pathogens like Fusarium oxysporum, Rhizoctonia spp., and Pythium debaryanum [45]. Bio-composts derived from various materials can be enriched with biocontrol agents capable of suppressing soil-borne plant pathogens. Applying such bio-composts shows promise for environmentally friendly pathogen control, enhanced soil fertility, and overall environmental sustainability.

Furthermore, as highlighted in recent studies, integrating biocontrol agents into bio-composts offers a promising avenue for enhancing pathogen suppression [47]. Such strategies align with the broader goals of sustainable agriculture, which seeks to reduce reliance on chemical inputs, improve soil health, and foster environmentally responsible farming practices. As we continue to explore the potential of bio-composts and their applications, we move closer to achieving agricultural systems that are both productive and ecologically sustainable.

Conclusion

In conclusion, the research highlights the substantial impact of leaf-based bio-compost amendments on soil microbial richness, diversity, and overall health. These amendments enhance microbial diversity and functionality, fostering beneficial microorganisms crucial for nutrient cycling, plant growth, and soil resilience. Leaf-based bio-composts increase beneficial bacteria and fungi while reducing pathogenic microorganisms, showcasing their potential for sustainable agriculture. Additionally, these bio-composts play a vital role in the bioremediation of soil-borne pathogens, further enhancing soil health and plant protection. The findings emphasize bio-composts as a sustainable solution for boosting agricultural productivity while mitigating the adverse effects of chemical inputs. By supporting soil health and ecosystem functioning, bio-composts align with sustainable agriculture principles, offering a promising approach to address environmental challenges and promote ecologically responsible farming practices. As research continues to uncover the potential of biocomposts, their application in agriculture holds significant promise for a more sustainable and productive future.

Declarations
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Author's contributions

S.M. – Data compilation, analysis, data curation, and original manuscript drafting

P.S. - Data compilation, analysis, and data curation

A.A. – Conceptualisation and methodology

S.N. – Conceptualisation, experimental design and supervision.

All the authors edited and proofread the final manuscript.

Statement and Declaration

Data availability

Data will be made available on request.

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Conflict of interest/Competing Interest

The authors have no competing or conflict of interest to declare that are relevant to the content of this article.

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This article does not involve any studies conducted by the authors with human participants or animals.

Consent to Participate

Not applicable.

Consent to Publish

Not applicable.
References


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**Figures**
Figure 1

The mapping of the soil sampling sites for experimental set up.

Figure 2

Rarefaction Curve: 16S

Rarefaction Curve: ITS

Sequence Sample Size

Sequence Sample Size

Comparison of sequence richness among different groups.

Group 5  Group 1  Group 4

Group 6  Group 5  Group 4

Group 6  Group 5  Group 4

Group 6  Group 5  Group 4

Group 6  Group 5  Group 4

Comparison of sequence richness among different groups.
Rarefaction curve of 16S and ITS metagenomics. The rarefaction curve indicates the sequencing depth of the samples, which also correlates with the species richness of the microbiome present in each sample. Group 1 includes the pre-plantation floodplain soil samples. YC, YD, YK, YM, YV, and YF; Group 4 includes the post-harvest floodplain soil samples. YCRAH, YDRAH, YKRAH, YMRAH, YVRAH, and YFRAH; Group 6 includes the residential post-harvest soil samples. NCRAH, NDRAH, NKRAH, and NFRAH.

Figure 3
Alpha diversity of the bacterial and fungal microbiome identified in the soil samples amended with different bio-composts. Group 1 is the pre-plantation soil samples of the Yamuna floodplain. Group 4 is the post-harvest soil samples of the Yamuna floodplain. Group 6 is the post-harvest residential soil samples. The pre-plantation soil samples had a higher microbial diversity than the post-harvest soils. The alpha diversity indices were significantly different among all the soil samples.

Figure 4

The beta diversity of the bacterial microbiome identified in the soil samples amended with different organic waste composites before plantation and post-harvest.
Figure 5

Heatmap of core bacterial genera present in the soil samples. The levels of bacterial genera identified in different soil samples varied depending on the type of compost amendment as the crop plantation.
Figure 6

The prominent beneficial bacterial genera identified in pre-plantation and post-harvest soils amended with different composts.
Figure 7

Heatmap of core fungal genera present in the soil samples. The levels of bacterial genera identified in different soil samples varied depending on the type of compost amendment as the crop plantation.
Figure 8

The prominent beneficial fungal genera identified in pre-plantation and post-harvest soils amended with different composts.

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